Influence of human activity on the stress-hormone levels and nestling growth of wild breeding Tree swallows (*Tachycineta bicolor*)

by

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Abstract

Anthropogenic disturbance can influence the physiology of individuals in wild breeding populations and have consequences for population ecology and conservation biology. The response to stressors in daily life is mediated by the hypothalamo-pituitary-adrenal (HPA) axis and can be assessed via concentrations of circulating glucocorticoids. Shortly after exposure, blood concentrations of glucocorticoids increase in response to environmental, social and internal stressors, enabling animals overcome a threatening situation. I tested the hypothesis that level of anthropogenic disturbance influences glucocorticoid concentrations in wild tree swallow nestlings. Across the field site, I qualitatively categorized nests as experiencing high or low levels of human activity. Additionally, I used an experiment in which adult females were repeatedly handled by humans during the time they reared nestlings. Just prior to fledging, I measured morphology and corticosterone (CORT) concentrations from the blood. Results did not match predictions: nestlings that experienced high human activity showed a reduced CORT response in addition to being smaller than nestlings in low activity areas. Since CORT was measured from blood taken 15 minutes after handling, it is possible that nestlings in high activity experienced decreased acute stress response as a result of chronic stress exposure. Repeated handling of mothers had no influence on nestling parameters suggesting effects of anthropogenic disturbance may be a consequence of maternal deposition of CORT in egg yolks. Overall, these data indicate complex interactions between human activity patterns and acute versus chronic stress in nestling Tree swallows.

Introduction

The nestling stage of life is a crucial time of development in avian species. For most altricial species, nestling development strongly influences the timing of fledging (Michaud and Leonard 2000) and by fledging earlier the risk of predation decreases (Götmark 2001). Nestlings are entirely reliant on parental care in terms of both food provisioning and predator protection; in most cases, parents continue to provision and protect young just after fledging (Ricklefs 1979). For most passerines, maximum body size is reached and fledging occurs around an age of 10-20 days (Ricklefs 1979). Developmental progress of nestlings is influenced by environmental factors (temperature, resource availability, etc.), but is more heavily influenced by parental provisioning. If development from nestling to fledgling is at all delayed or stalled in an individual, this increases the likelihood of mortality or lead to long-lasting effects on the body condition, if it does not lead to nestling mortality (McCarty 2001; Lendvai et al. 2009; Stöwe et al. 2010). Reduced body condition and increased likelihood of mortality can result from environmental stressors faced by the nestling, such as lack of resources and brood size (Lendavi et al. 2009). Food availability and threat of predation can influence how parents provision and defend offspring (Love et al. 2013; Storm et al. 2010).

In vertebrates, the hypothalamo-pituitary-adrenal (HPA) axis is responsible for how an individual responses to a stressful situations, and influences an individual's survival in changing environments (Boonstra 2004; Specner et al. 2009). Exposure to stressful situations may lead to the release of corticosterone (CORT), a glucocorticoid or stress-hormone, in an individual. There are two stress levels one must take into account: baseline and elevated. Baseline stress relates to the daily CORT level of an individual when not faced with a stressor. Elevated stress is the CORT concentration found when one is exposed to a stressor (Bókony et al. 2009).

Parents, in order to ensure offspring success in the face of potential nest predators or nest usurpers, are likely to show aggressive behavior. However, such aggression can cause stress to offspring, especially if the aggression is mainly maternal (Love et al. 2013). The anti-predator response is one of the most common stress responses in songbirds (Thompson and Burhans 2003). When faced with even just a potential threat, birds are likely to alter habitat use as well as reproductive effort (Thompson and Burhans 2003). Mothers tend to be highly aware of and proactive towards potential nest threats and warn offspring. This is likely a proximate mechanism in which mothers can mediate offspring stress levels or response of offspring to stress and, perhaps, better prepare offspring for predation threats (Storm and Lima 2010). In songbirds, females could influence offspring stress response by provisioning or anti-predator behaviors during the nestling stage (Hayward and Wingfield 2004) or directly via 'maternal effects' during egg formation (Bentz and Siefferman 2013). Environmentally-induced variation in egg hormones is a classic example of a maternal effect, which is a non-genetic mechanism whereby the conditions breeding females experience can influence offspring phenotype (Groothuis et al., 2005; Wolf and Wade, 2009).

Stress to an individual can be classified as acute and chronic. Acute stress occurs when there is a temporary stressor versus chronic stress when there is a constant stressor present, typically in relation to the environment. For nestlings, parental provisioning or anti-predator behaviors and temporary food stress could induce acute stress while elevated CORT in egg yolks and extended periods of time under food stress, or other environmental factors, could induce chronic stress (Blas et al. 2005). Bodily reactions to stress vary based on the type of stress present. In nestlings, exposure to stress not only elevates stress via the release of CORT but also impacts development and leads to prolonged physiological acute stress later in life (Spencer et al. 2009). Pickering et al. (1991), they found that acute stress decreases the growth hormones in rainbow trout whereas chronic stress increased the hormone levels. Though birds have a different growth and development than fish, this study indicated that chronic stress could lead individuals to reach maturity faster in order to avoid predation.

Currently, the threat most often faced by populations is human encroachment (Wilson and Wilson 1975). This can be seen via direct development or general habitat encroachment in a region. Crino et al. (2013) found that environmental stressors, in the form of traffic noise, on white-crowned sparrow nestlings increased their stress hormone levels, while decreasing overall growth and immune functions. Additionally, human encroachment via bird watching and calling has been shown to cause stress to mating pairs (Sekercioglu 2002). These are just a few studies to indicate the potential impacts of human activity on wild populations.

By studying how parent Tree swallows (*Tachycineta bicolor)* respond to human activity, researchers can understand how human encroachment influences nestling development and stress levels. Here I use both the natural variation in human activity and an experiment (of repeatedly handling breeding females) to test the effect of anthropogenic disturbance on nestling physiology. This approach should elucidate whether there is a greater influence of acute stress on mothers (handling study) or chronic stressors to nestlings (human activity) on nestling development. I predict that mothers that were repeatedly handled will have offspring that show differential development and increased levels of stress hormones.

Further, I predict that nestlings reared in locations of high human activity will exhibit increased CORT levels and decreased body condition.

Methods

Study Species

Tree swallows are socially monogamous secondary cavity nestling passerines that readily nest in artificial cavities (Winkler et al. 2011). Tree swallows exhibit bi-parental care, meaning both participate in the care of young. Along with food provisioning for nestlings, parents tend to be aggressive towards any predators or nest usurpers (both con- and hetero-specifics) (Winkler et al. 2011). Tree swallows breed from early May to late June, produce one nest per year, lay 4-7 eggs, and females incubate young for 11-20 days. Nestlings fledge the nest between ages 15-25 days (Winkler et al. 2011).

Study Site

The study was conducted in Watauga Co., NC (latitude: 36.283, longitude: -81.627) from May through July 2013. The field site has 170 nest boxes that have been monitored since 2009. All nests were monitored for laying date, clutch size, and hatch date.

Maternal Handling Experiment

A study was conducted to manipulate human handling of breeding female Tree swallows to understand how multiple handling by humans influences parental defense of young (from mock predators) and parental feeding rates to young. Female Tree swallows were randomly assigned to one of two groups: control and treatment. Females in the control group were captured only once (when nestlings were ~7 days old) and the treatment was caught four times (when nestlings were between 0-7 days old). When nestlings were 10 days old mock predators (model crows) were placed 0.3m above the nest boxes to elicit a defensive response from the parents. Timing and frequency of parental attacks were recorded as a measure of parental aggression. Females that were repeatedly handled were more aggressive towards mock predators (L. Siefferman, unpublished data). When nestlings were 8 days old, parental provisioning rates to nestlings were measured using video cameras. Females that were repeatedly handled fed offspring more often (L. Siefferman, unpublished data). The handling experiment did not influences parental defense or provisioning behavior of the female's mates (L. Siefferman, unpublished data). Because the experiments were conducted after egg-laying, the experiment would not have influenced yolk hormone levels and any influence of the experiment on nestling physiology should have occurred due to changes in parental care behaviors. For analysis of maternal influence on nestling stress and body condition, mothers were categorized based on their capture status (single capture (n=27) versus recapture (n=18)).

Variation in Human Activity Across the Field Site

Each nest box was given a numerical value to qualitatively represent the natural level of human activity near the nest box. A ranking of 1 is defined as low to moderate activity, meaning people occasionally passing the nest, but should not have been creating a constant, significant disruption. A ranking of 2 is defined as high levels of human activity near the nest box, such as parks (Greenvalley Community Park, Todd Island Community Park, and along Railroad Grade Road in Todd, NC). These locations experience moderate to high recreational activity (fishing, walking, bicycling, river tubing). There were 28 nests assigned a value of 1 and 17 nests assigned a value of 2.

Nestling Growth Measurements

Individual nestlings in each nest were identified at age 2 days (hatch day = day 1)and marked using non-toxic Sharpie® marker and then were banded with USGS numbered bands at age 8 days. Nestling mass was measured at ages 8 and 14 days. Wing length of day 14 nestlings was measured. Tree swallow mass reaches an asymptote at approximately age 13 days, while wing feathers continue to grow until age 30 days (Winkler et al. 2011). For Tree swallows, nesting morphology at age 14 is a good representation of final adult mass while wing length is a good proxy of flight ability at fledging (Winkler et al. 2011).

Hormone Data Collection

At age 14 days, 80ul blood samples were collected via puncture of the brachial vein of nestling 15-25 minutes after initial handling. Adequate volume of blood was collected from 84 nestlings from 46 broods. Blood plasma and red blood cells were separated using centrifuge and plasma was retained for CORT assays and held at -80°C. CORT assays were measured by Alexandra Bentz (University of Georgia). Serum CORT was extracted using celite column chromatography following methods modified by Schwabl (1993). Briefly, 20ul of serum was mixed with 3ml dietyl ether, vortexed and allowed to settle for 20min. Samples were snap frozen and the liquid portion containing CORT was reserved and dried using N_2. Samples were re-suspended in 1 mL of 10% ethyl acetate in isooctane and then eluted through columns in varying fractions of ethyl acetate in isooctane. Samples were dried using N_2 and CORT quantified using a competitive binding radioimmunoassay following Wingfield and Farner (1975).

Nest Box Occupancy

To test whether human activity was associated with preferred habitat of Tree swallows, I used data collected from 2009-2013 to quantify how often nest boxes were occupied by breeding tree swallows. The assumption is that boxes that were occupied at a higher rate are higher quality habitat (Jones et al. 2014).

Data Analysis

Data was analyzed with IBM SPSS Version 22. A series of linear mixed models were used to test the influence of the independent variables (handling experiment and human activity levels) on the dependent variables (CORT levels, nestling mass and wing length). CORT data were log transformed. Nestling mass and wing data were standardized (mean=0). In all analyses, nest ID was used as a random factor to control for the non-independence of multiple nestlings per brood. A t-test was run to examine whether level of human activity was associated with nest box occupancy rates. Alpha was set at $p \leq 0.05$.

Results

Maternal Handling Experiment and Nestling Parameters

I used linear mixed models to test the significance of the human handling experiment on nestling growth parameters (mass and wing length) and CORT levels. Human handling did not significantly influence CORT, nestling mass or wing at day 14 (Table 1).

Human Activity and Nestling Parameters

I used a linear mixed model to determine whether human activity level was associated with nestling growth (mass and wing length) and CORT level. Nestling mass and CORT was significantly greater when nestlings were reared in locations of low human activity (Table 2; Figure 1, Figure 2). Human activity, however, was not significantly associated with nestling wing length (Table 2).

Covariation between Nestling Parameters

Pearson correlations revealed no significant relationship between nestling mass and CORT levels ($r = -0.027$, $n = 85$, $p = 0.809$; Figure 3). Nestlings with longer wings had significantly greater mass ($r = 0.365$, $n = 162$, $p < 0.001$). There was no significant relationship between nestling wing length and CORT levels ($r = -0.154$, $n = 84$, $p = 0.161$).

Nest Occupancy and Human Activity

I found no significant association between nest occupancy and human activity (mean (+/- 1 SD) occupancy in low human activity locations: 0.591 (+/-0.257); mean occupancy in high human activity: 0.706 +/- 0.214; $t = 1.546$, $n = 45$, $p = 0.130$).

Discussion

Overall, I found that natural variation in human activity plays influenced nestling mass and stress levels while I found no influence of the handling experiment on nestling growth parameters. Natural variation in human activity spanned across the site during settlement, egg laying and nestling rearing, while the maternal handling experiment only occurred during the nestling stage. These data suggest that human encroachment during egg laying may be more important to nestling stress profiles and growth than parental behavior during the nestling stage. Nestlings reared in locations with greater human activity exhibited lower mass at fledging-which was expected, however, those nestlings also exhibited reduced CORT levels. Moreover, across the population, larger chicks responded with higher CORT levels. There are two possible explanations to these findings: reduced acute stress response as a result of chronic stress or maternal deposition of CORT into the yolk.

CORT levels for this study were measured 15-25 minutes after nestling handling, representing elevated, rather than baseline glucocorticoid levels. Therefore, the nestlings in areas of high activity may be exhibiting a reduced acute stress response because they have been exposed to chronic environmental stress (Figure 2). A study by Katz et al. (1981) found that a history of chronic stress reduces ones ability to appropriately respond to acute stressors later in life. Stress variation is key in order for an individual to better respond to an environmental change (Boonstra 2004). The Tree swallow nestlings exhibiting high CORT levels may experience reduced fitness if they are unable to appropriately respond to various situations, such as predation threats, later in life (Katz et al. 1981).

Alternatively, or additionally, these results could be indicative of maternal stress at the time of egg laying, leading to the deposition of excess CORT into the yolk (Hayward and Wingfield 2004). This would be classified as a chronic stressor throughout a nestling's life and would elevate baseline, or homeostatic, CORT levels. Again, elevated baseline CORT, as a result of a chronic stress, may reduce a nestling's ability to appropriately respond to acute stress. Though I found no evidence that maternal handling influenced nestling CORT, exposure to CORT before hatching via the mother is possible.

The nestlings in the regions of low activity not only exhibited greater levels of CORT, but also showed a greater body mass at the time of fledging (Figure 3). If the difference between baseline and elevated CORT is a key factor in the results, then it is possible to say nestlings exposed to chronic stress have a reduced physiological condition. The correlation seen between greater mass and higher CORT could be explained as a relationship mass and response to an acute stress (Figure 3). Various nestling studies have shown a relation between chronic stress and reduced body condition, which would explain the connection between these factors (Stöwe et al. 2010; Crino et al. 2013). In retrospect, this study would have been better designed if baseline, rather than elevated, CORT levels were measured. The results for CORT were opposite of what I predicted, however they could be explained as a reduced stress response caused by chronic stress exposure.

Wing length was not significant for either test, likely because the wings still have time to fully develop; the timing of full wing development is indicative of when the nestlings fully fledge and leave the nest (Michaud and Leonard 2000).

Although I qualitatively classified the field site as experiencing high or low human activity, other aspect of habitat may covary with human activity patterns. It is possible that areas of higher human activity are lower quality habitat for tree swallows in general these locations may have lower prey availability or quality. Therefore, I used a long-term measure of habitat preference to investigate covariance between habitat preferences and human activity patterns. Using data from 2009-2013, I calculated nest box occupancy with the assumption that boxes that were used more often by Tree swallows represent high territory quality. However, nest box occupancy was not significantly related to human activity patterns, thus it seems unlikely that habitat preference, habitat quality or competition for nest boxes influenced the relationships I found between nestling parameters and human activity patterns.

Future Research

To better understand the relationship between human activity and nestlings stress and growth, future work should focus on baseline rather than elevated CORT. This study would lead to greater understanding as to whether or not human presence influences the fitness of Tree swallows as a result of anthropogenic stress. To gain a better understanding of stressors and growth, a study that manipulates human activity level would be helpful. By altering stress exposure, one could better collect quantitative data related to activity rather than the qualitative measures used in this study.

Furthermore, studying the preferred habitat of Tree swallows could determine the overlap of tree swallow populations and high human recreation areas. Habitat quality could be studied via nest box occupation and prey studies. Quality and variety of prey in a region could give a good approximation of where Tree swallows congregate as well as where one is likely to find high conspecific and other passerine competition for resources. Identifying areas of high completion could lead to the understanding of another stress source that influences nestlings.

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Table 1: Effect of human handling of mothers (during the nestling stage) on nestling Tree swallow morphology and corticosterone (CORT) at age 14 days post hatch.

Factors	**df**	**Est (+/- 1SE)**	***F***	***P***
Mass (g)	33.625	-0.066(0.242)	0.074	0.787
Wing Length (mm)	31.494	0.297 (0.268)	1.225	0.277
CORT (log ng/ml)	33.343	-0.059 (0.086)	0.469	0.498

The parameter of recaptured individuals was set to 0.

Table 2: Effect of human activity levels on nestling Tree swallow morphology and corticosterone (CORT) at age 14 days post hatch.

Factors	**df**	**Est (+/- 1SE)**	***F***	***P***
Mass (g)	34.828	0.563 (0.240)	5.491	0.025
Wing Length (mm)	32.130	0.004 (0.290)	0.000	0.989
CORT (log ng/ml)	33.524	0.259 (0.078)	10.992	0.002

The parameter of human activity ranking of 2 was set to 0.

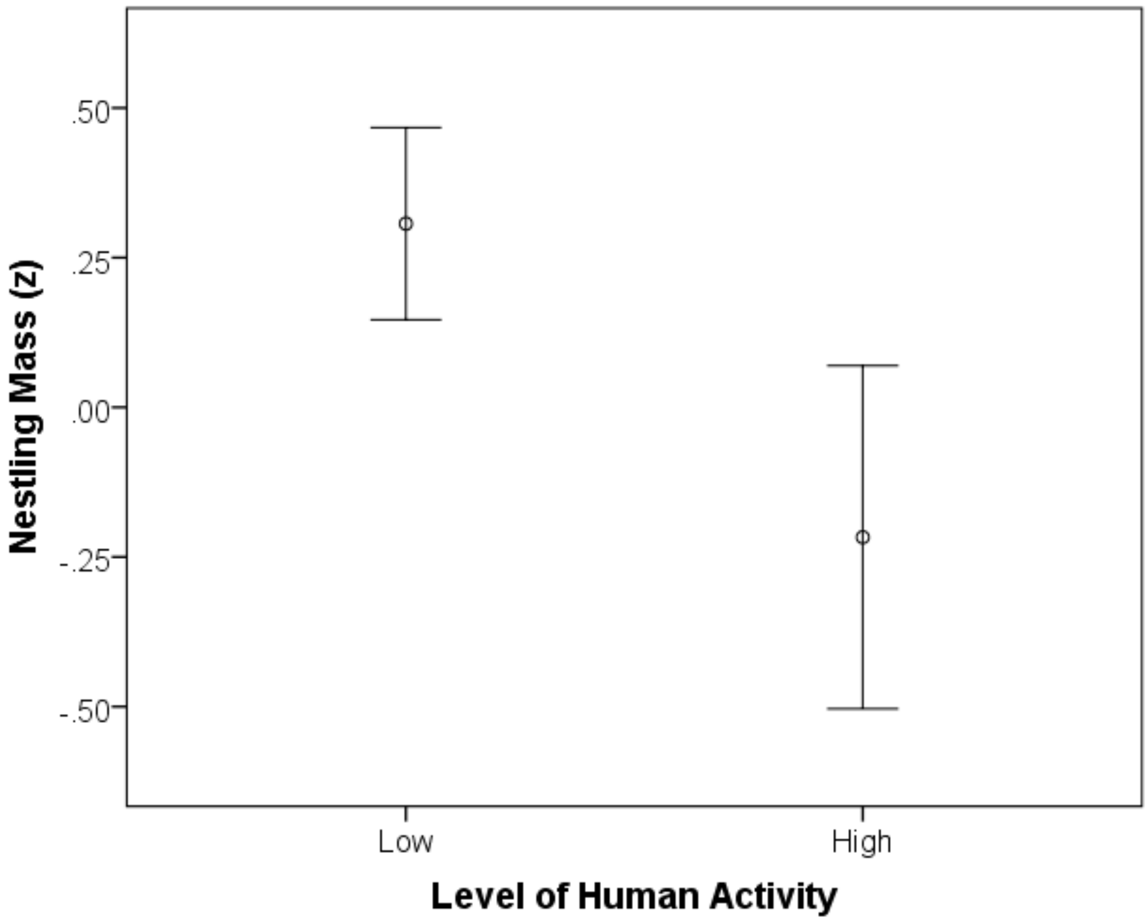

Figure 1: Relationship between natural variation in human activity and standardized nestling mass of day 14 Tree swallow nestlings.

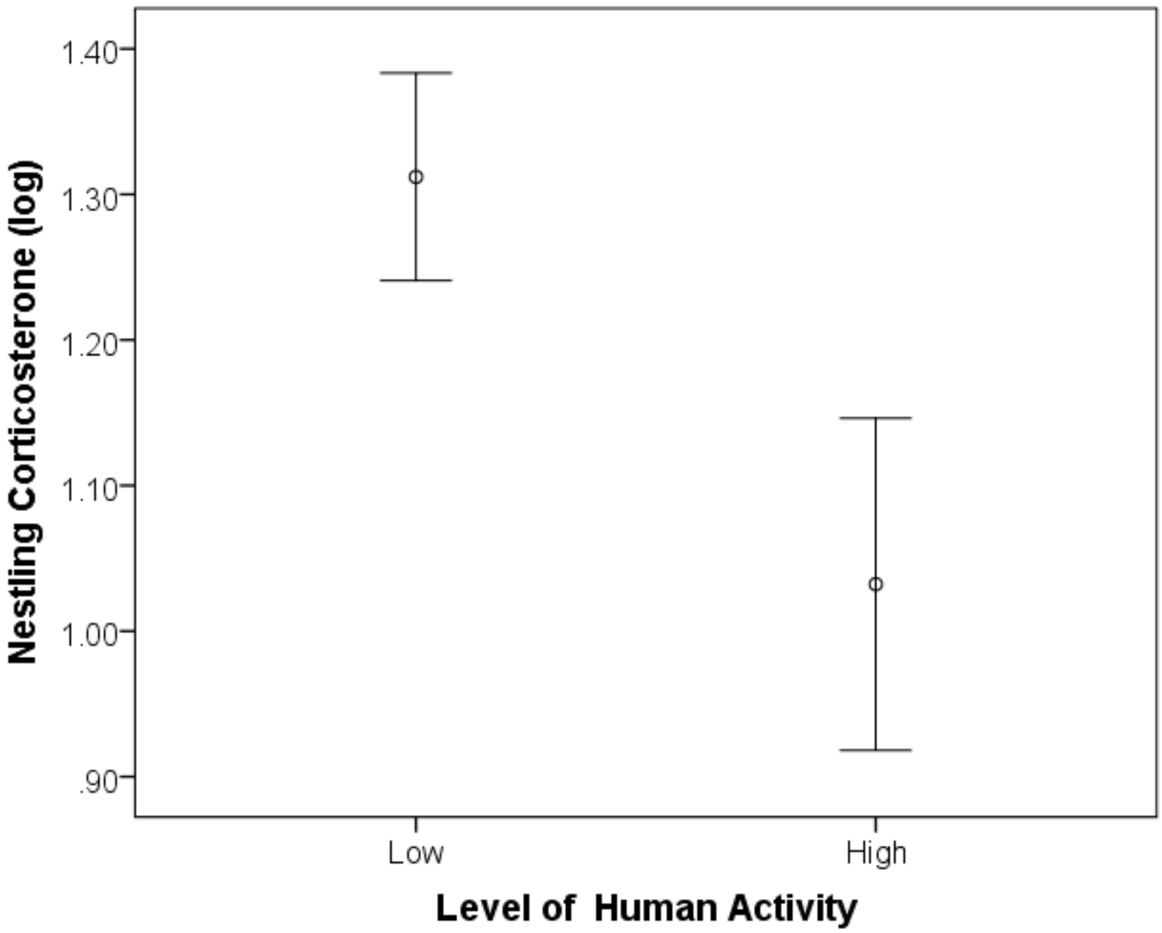

Figure 2: Relationship between natural variation in human activity and log transformed corticosterone levels of day 14 Tree swallow nestlings.

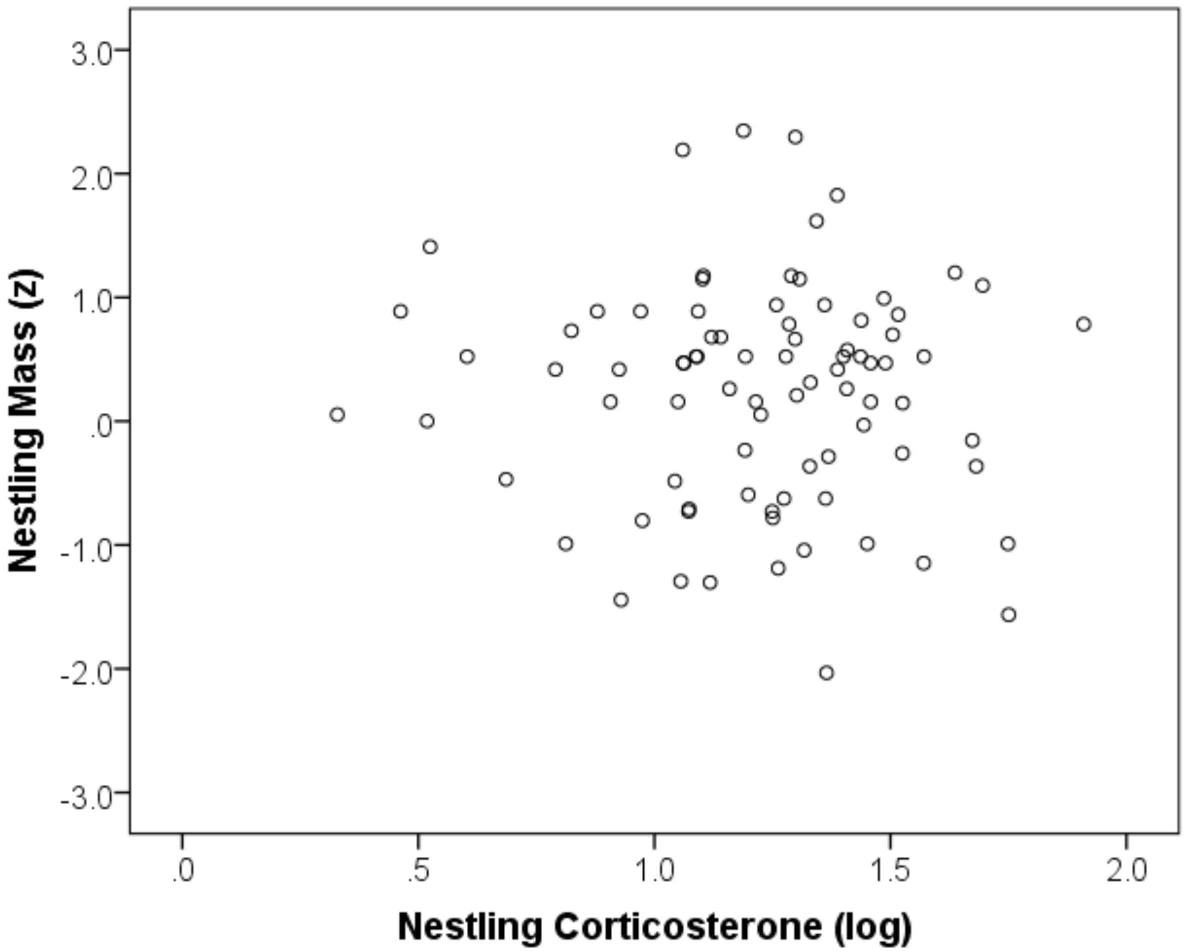

Figure 3: Relationship between nestling mass (standardized) and corticosterone (log transformed) levels of day 14 nestling Tree swallows.